Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Environmental Sciences 11 (2011) 1459 – 1464

Procedia Environmental Sciences

The Effect of O-Dihydroxybenzene and Pyrogallol on the Chlorella Pyrenoidosa Chick Growing

WU Shan,LI Xiao-hong ,YANG Long

The College of Architecture and Civil Engineering,Beijing University of Technology
Beijing, China
wushan@bjut.edu.cn

Abstract

The allelopathy of aquatic plants which can inhibit the excessive breeding of algae is a new water quality control technology which is low-carbon and green compared with the traditional treatment methods. In this experiment, choosing the content of algal cell's chlorophyll-a as an basic indicator, the allelopathy degree of different concentrations of O-Dihydroxybenzene and Pyrogallol on algal growth and metabolism is analyzed, and choosing A_{560nm} as an indicator, the influence of different concentrations of O-Dihydroxybenzene and Pyrogallol on superoxide dismutase activity of *Chlorella Pyrenoidosa Chick* is analyzed. And to explore the relationship of allelopathy and superoxide dismutase activity, the results could be used to provide an experimental basis for the technology of allelopathy on the growth of algae control.

*Keywords:*Allelopathy, o-dihydroxybenzene, pyrogallol, Chlorella Pyrenoidosa Chick, superoxide dismutase, chloroplast-a

1. Introduction

The allelopathy of aquatic plants which can inhibit the excessive breeding of algae is a new water quality control technology which is low-carbon and green compared with the traditional physical and chemical treatment methods. For no chemical dosing needed and no energy consumption as well as less damage to the environment, it could get integrated benefits both for water quality environment and socio-economic.

With the discovery of and research on allelopathic effect of water plants on the algae in recent years, allelopathy has gradually become an important means for controlling water eutrophication. As early as in 1949, Hasler et al found that water plants exert suppression effect on algae. Allelopathic materials suppress algae's growth mainly through the following mechanisms [1-3]: influence photosynthesis; influence enzyme activity; disrupt cell film; influence aspiration and metabolization; suppress nutrition absorption; change sub-micron structure, and etc. Allelopathic materials can act through one or several of the above-mentioned mechanisms.

doi:10.1016/j.proenv.2011.12.219

Organisms have formed an anti-oxidization enzyme system composed of superoxide dismutase (hereafter SOD for short), peroxidase (POD) and catalase (CAT) , the three of which can eliminate active oxygen, thus maintaining dynamic balance of the organisms' oxygenic metabolization and protecting the organisms[4~6]. SOD enzyme is the first one that enacts elimination of active oxygen by eliminating O_2^- (free radical of superoxide anion); and through the catalytic reaction ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$), the outcome H_2O_2 can be further decomposed and utilized by peroxidase and ascorbic acid [7]. Therefore, SOD acts as the first line of defense. Activity of anti-oxidization enzyme within the organism reflects its growth condition.

O-Dihydroxybenzene and Pyrogallol are two allelopathic materials that have been proven to exert allelopathic influence on maeruginosa, but their suppression effect on other algae and their concentration for the best suppression effect have jet been reported[8-9].

Choosing as the experiment object *Chlorella Pyrenoidosa Chick*, an oft-seen alga in the scenic water of Beijing city, during the experiment O-Dihydroxybenzene and Pyrogallol were added in the medium with different concentrations to measure chloroplast-A content and SOD activity, thus analyzing allelopathic influence of O-Dihydroxybenzene and Pyrogallol on specific alga and the concentration for the best suppression effect within the given concentration scope. Meanwhile, relationship between allelopathic suppression effect and SOD activity will be analyzed to provide experimental reference for suppressing algae through allelopathy. The results could be used to provide an experimental basis for the technology of allelopathy on the growth of algae control.

2. Material and Methods

2.1.Materials Selecting

Alga and culture medium： *Chlorella Pyrenoidosa Chick* is provided by Wuhan Institute of Hydrobiology, Chinese Academy of Sciences. The culture medium is based on SE culture medium and a comparison will be made between medium without allelopathic materials and those added by allelopathic materials with different concentrations. Before the experiment, the alga is on the stage of logarithmic growth through pre-culture. During the experiment culture, the ratio of lightness to darkness is controlled at 14h：10h and the culture temperature is kept at 25℃.

Allelopathic materials: O-Dihydroxybenzene and Pyrogallol.The solution concentration is 0.0001g/ml.

2.2. Methods

Add 195 ml SE culture medium and then solution with different concentrations of allelopathic materials (O-Dihydroxybenzene and Pyrogallol) in a series of 250 ml conical bottles after destroying the bacteria. The contents of allelopathic materials are 0ml, 1ml, 2ml, 4ml and 8mL to produce alga culture solution concentration of 0mg/L, 0.5mg/L, 1mg/L, 2mg/L and 4 mg/L respectively. 2 parallel samples are prepared for each group. Each time, 8ml alga solution will be drawn to go through centrifugation for 20 minutes at the speed of 5000r/min; after removing the clear liquid on the upper part, the solution will be added with 1ml cooled phosphoric acid buffer solution (concentration: 0.05mol/L, pH=7.4) to be grinded into seriflux in electric homogenizer on the ice water (0-4℃). Add buffer solution to the seriflux to make 5ml solution which will go through centrifugation for 20 minutes at the speed of 7000r/min. The clear liquid on the upper part, i.e. the coarse enzyme solution, is used to measure the enzyme activity [4].

2.3.Analysis and Measurement Methods

Chloroplast-A is measured in accordance with *Monitoring and Analyzing Method for Water and Wastewater* (4^{th} edition).

SOD enzyme activity is measured in accordance with NBT methods, which determine the enzyme activity according to SOD's suppression on NBT's light reduction: when oxide exists, riboflavin will be reduced by light and the reduced riboflavin is very easy to produce oxygen ion O_2^- under the existence of

oxygen and O_2^- can reduce NBT to blue methylamine which reaches maximal absorption at 560 nm; while SOD can eliminate O_2^- and thus suppress formation of methylamine. Therefore, under light reduction, the reaction solution color will become heavier and the light absorption quicker, which means that the enzyme activity is lower; otherwise the activity is higher [10]. The reaction system is as follows: 1.5 ml phosphoric acid buffer solution (concentration: 0.05mol/L, PH=7.4), 0.3 ml Met solution (130mmol/L), 0.3 ml NBT solution (750umol/L), 0.3 ml EDTA-Na_2 solution (100umol/L), 0.3 ml riboflavin (20umol/L), 0.05 ml enzyme solution and 0.025 ml distilled water.

3. Results and Discussion

3.1.Influence of O-Dihydroxybenzene and Pyrogallol on the Growth of Chlorella Pyrenoidosa Chick

1) Chloroplast-a Experiment

Figure 1 shows that during the whole culture period, chloroplast-a is employed as the index to reflect influence of *O-Dihydroxybenzene* with different concentrations on the growth of *Chlorella Pyrenoidosa Chick.*

As it is shown in the figure, from very beginning of culture the alga's growth rate in each group added with *O-Dihydroxybenzene* evidently decreases lower than the comparison group, meaning that allelopathic effect is very evident. Later, growth rate in these groups are lower than that in the comparison group until the culture ends. Viewed from the final result, the suppression effect reaches its summit when *O-Dihydroxybenzene*'s concentration is 2mg/L. When the culture ends, chloroplast-a content in the comparison group is 429.77mg/m^3, while the one in the 2mg/L *O-Dihydroxybenzene* group is 167.03mg/m^3. Calculated by the chloroplast-a content, the suppression rate is 61%.

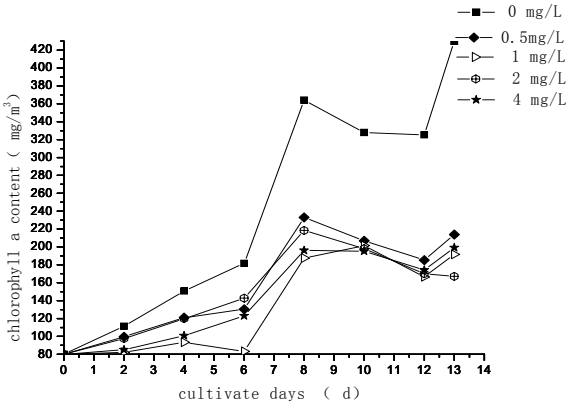

Figure 1. Effect of o-dihydroxybenzene on content of chlorophyll-a of *Chlorella Pyrenoidosa Chick*

2) SOD Activity Experiment

Figure 2 shows that during the whole culture period, A_{560nm} is employed as the indicator to reflect influence of *O-Dihydroxybenzene* with different concentrations on the SOD activity of *Chlorella Pyrenoidosa Chick*.

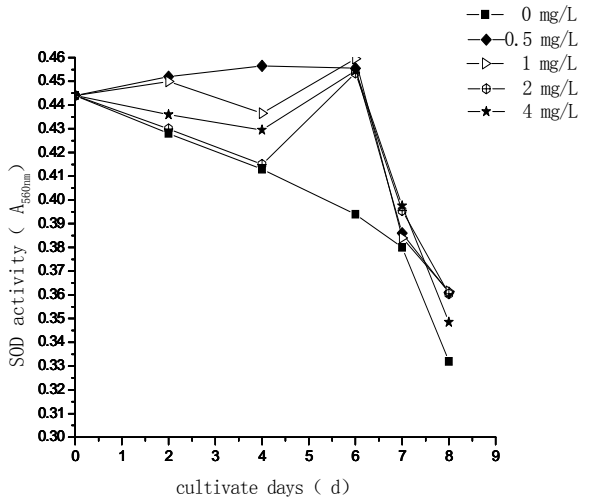

Figure 2. Effect of o-dihydroxybenzene on SOD activity of Chlorella Pyrenoidosa Chick

The higher the value of A560nm is, the lower the enzyme activity is. When A560nm value in the comparison group decreases, it means that SOD enzyme activity increases. Since the alga grows with the time, the number of SOD increases and the enzyme activity increases accordingly. It is from the beginning of the culture that influence on the SOD enzyme activity of Chlorella Pyrenoidosa Chick has become evident. At the end of the culture, the value of A_{560}nm is almost the same with the o-dihydroxybenzene's concentration are respectively 0.5mg/L, 1mg/L and 2mg/L. That means the SOD activity is also very close.

3.2.Influence of Pyrogallol on the Growth of Chlorella Pyrenoidosa Chick

1) Chloroplast-a Experiment

Figure 3 shows that during the whole culture period, chloroplast-a is employed as the index to reflect influence of *Pyrogallol* with different concentrations on the growth of *Chlorella Pyrenoidosa Chick.*

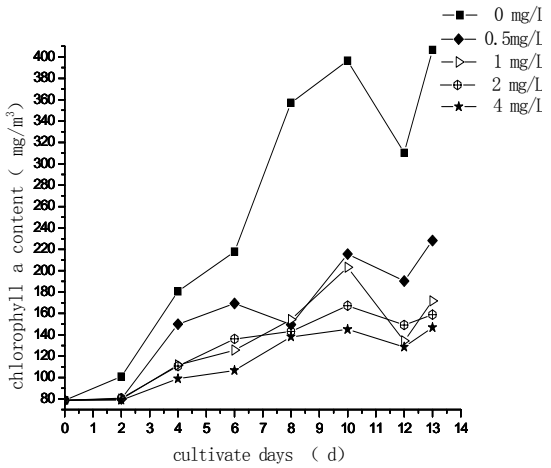

Figure 3. Effect of pyrogallol on content of chlorophyll-a of Chlorella Pyrenoidosa Chick

Similarly with O-dihydroxybenzene, Pyrogallol's suppression effect is very evident even from the beginning of the culture, and change trend of the suppression effect is almost the same with each of these groups. Viewed from the final result, the suppression effect is most evident when Pyrogallol's concentration is 4mg/L. When the culture ends, chloroplast-a content in the comparison group is 406.60mg/m^3, while the one in the 4mg/L Pyrogallol group is 146.81mg/m^3. Calculated by the chloroplast-a content, the suppression rate is 63.9%.

2) SOD Activity Experiment

Figure 4 shows that during the whole culture period, A_{560nm} is employed as the indicator to reflect influence of pyrogallol with different concentrations on the SOD enzyme activity of *Chlorella Pyrenoidosa Chick*.

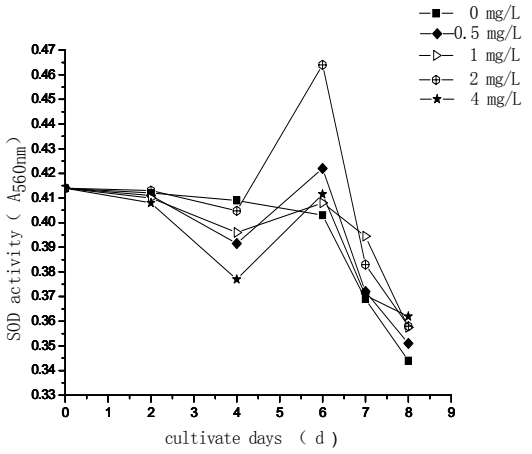

Figure 4. Effect of pyrogallol on SOD activity of Chlorella Pyrenoidosa Chick

When A_{560nm} value in the comparison group decreases, it means that SOD enzyme activity increases. Since the alga grows with the time, the number of SOD increases and the enzyme activity increases accordingly. As it is shown in the figure 4, unlike with O-Dihydroxybenzene, Pyrogallol's influence on SOD activity of *Chlorella Pyrenoidosa Chick* is shown after the 6th culture day, the A_{560nm} value in each group added with Pyrogallol evidently higher than the comparison group, meaning the SOD enzyme activity decreases.

A_{560nm} value is highest when the *Pyrogallol*'s concentration is 4mg/L, which means that SOD activity is lowest. The SOD activity increases gradually with 4mg/L, 2mg/L, 1mg/L and 0.5mg/L, 0 mg/L (*Pyrogallol* content) .

4. Conclusions

- Chloroplast-a serves as the index to reflect that both O-Dihydroxybenzene and Pyrogallol suppress Chlorella Pyrenoidosa Chick, and they both shown suppression effect from the beginning of the culture, and change trend of the suppression effect is almost the same with each of group. The difference is they achieve their best suppression effect respectively in the concentration of 2mg/L (O-Dihydroxybenzene) and 4mg/L (Pyrogallol).
- Different from influence on chloroplast a, influence of *O-Dihydroxybenzene* and *Pyrogallol* on SOD activity of *Chlorella Pyrenoidosa Chick* shown in difference culture days: in the sixth day for *O-Dihydroxybenzene* and from the beginning for *Pyrogallol.*
- Chloroplast a serves as the indicator to reflect that O-Dihydroxybenzene suppress *Chlorella Pyrenoidosa Chick*, but that when the culture ends, change of the suppression effect does not follow the same concentration order with the effect on SOD activity. It can be deduced that effect of *O-*

Dihydroxybenzene on *Chlorella Pyrenoidosa Chick* does not merely come from suppression of enzyme activity, but also from other suppression contributors.

- Compared with the results of Pyrogallol's suppress effect by Chloroplast-a and A_{560nm} as the indicator separately, it is found that they follow the same concentration order of Pyrogallol added in the culture medium. It can be deduced that effect of Pyrogallol on *Chlorella Pyrenoidosa Chick* basicly come from suppression of SOD enzyme activity.

References

[1] Wu Xiaohui.Studies on the allelopathy of several submerged Potamogetonaceae species on planktonic algae [D].Wuhan: Chinese Academy of Sciences .2005. (In Chinese)

[2] Li Fengmin,Hu Hongying,Zhong Yunxiao,et al. Effects of Allelochemical EMA Isolated from Phragmites communis on Algal Cell Membrane Lipid and Ultrastructure [J]. Environmental Science, 2007, 28(7):1534-8. (In Chinese)

[3] Hong Yu,Hu Hongying. Research and application of inhibitory allelopathy from aquatic plants on algae [J].Chinese Science Bulletin, 2009,54(03): 287-293. (In Chinese).

[4] Li Fengmin,Hu Hongying,Men Yujie,et al.Effects of EMA on Activities of Algal Antioxidant Enzymes [J]. Environmental Science, 2006, 27(10):2091-2094. (In Chinese).

[5] Peng Jinliang,Yan Guoan,Shen Guoxing, et al. The Effects of α-Naphthol on the Growth and the Antioxidase Activities of Chlorella Vulgaris [J]. Journal of wuhan University (Natural Science Edition), 2001, 47(4):449-452. (In Chinese).

[6] Oncel I, Yurdakulol E, Keles Y,et al. Role of antioxidant defense system and bio-chemical adaptation on stress tolerance of high mountain and steppe plants [J]. Acta Oecologica -International Journal of Ecology, 2004, 26(3): 211~218

[7] Zuo Xiangyu.Study on the Growth Characteristics and Antioxidant Enzymes Activities of Two Raphidophytes under Different Cultural Conditions [D].guangzhou, Jinan University, 2006:10~12. (In Chinese)

[8] Hu Hongying,Men Yujie,Li Fengmin. Research progress on phyto-allelopathic algae control [J]. Ecology and Environment, 2006, 15(1):153-157. (In Chinese)

[9] Hua Ming,Chen Liangyan,Yin Daqiang. The compare on the mechanism of pyrogallic acid and caffeic acid allelochemicals to algae (Microcystis aeruginosa) [J]. Environmental Chemistry, 2008, 27(3):331-334. (In Chinese)

[10] Wang Xuekui. Plant physiology and biochemistry experiment principle and technology (Second Edition) [M]. Beijjing: Higher Education Press, 2006. (In Chinese)